

Artikel Asli/Original Article

Fixatives Increase the Efficacy of Gel Formulations Containing *Piper aduncum* Linnaeus (Piperales: Piperaceae) Essential Oil as Repellent
(Fiksatif Meningkatkan Keberkesanan Formulasi Gel yang Mengandungi Minyak Pati *Piper aduncum* Linnaeus (Piperales: Piperaceae) Sebagai Repelan)

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ABSTRACT

Piper aduncum essential oil exhibit repellency activity and has a potential to be use as an alternative for synthetic repellent such as *N,N*-diethyl-3- methylbenzamide, (DEET). However, the volatility properties of the essential oil decrease their persistence as a topical repellent. Study has shown that formulation of the essential oil with some fixatives may increase their effectiveness. Therefore, this study was conduct to evaluate the effectiveness of gel formulation containing *P. aduncum* essential oil with two fixative; vanillin and paraffin oil. Gel formulations containing *P. aduncum* essential oil with 5% and 10% vanillin and 6% paraffin oil was prepared and tested against *Aedes aegypti* in laboratory using Standards and Industrial Research Institute of Malaysia (SIRIM) bioassay method. After 240 minute post-application, formulation containing 5% and 10% vanillin was able to provide >70% repellency percentage against mosquito while formulation containing 6% paraffin oil gives <30% repellency percentage. As conclusion, *P. aduncum* based repellent gel containing 5% and 10% vanillin was able to prolong the effect of *P. aduncum* essential oil as repellent against dengue vector in laboratory.

Keywords: Repellent; *Piper aduncum*; *N,N*-diethyl-3- methylbenzamide; gel; vanillin; paraffin oil

ABSTRAK

Kajian lepas telah menunjukkan bahawa minyak pati *Piper aduncum* mempunyai aktiviti repelan terhadap *Aedes aegypti* dan berpotensi untuk dijadikan alternatif kepada repelan sintetik seperti *N,N*-dietil-3- metilbenzamida, (DEET). Walau bagaimanapun, sifat kompaun aktifnya yang mudah meruap telah menyebabkan keberkesanannya sebagai repelan hanya bertahan dalam jangka masa yang singkat. Penambahan bahan fiksatif ke dalam formulasi yang mengandungi minyak pati didapati dapat meningkatkan keberkesanannya sebagai repelan. Oleh sebab itu, kajian ini telah dijalankan untuk menilai keberkesanan formulasi gel yang mengandungi minyak pati *P. aduncum* dan dua jenis bahan fiksatif iaitu vanillin dan minyak parafin sebagai repelan. Formulasi gel telah disediakan dan kemudian diuji keberkesanannya terhadap *Ae. aegypti* di makmal berpandukan kaedah Institut Piawaian dan Penyelidikan Perindustrian Malaysia (SIRIM). Selepas 240 minit pos-aplikasi, formulasi yang mengandungi 5% dan 10% vanillin dapat memberikan >70% peratus penangkisan terhadap *Ae. aegypti* manakala formulasi gel yang mengandungi 6% minyak parafin hanya mampu menangkis <30% populasi nyamuk. Secara kesimpulannya, kajian mendapati bahawa penambahan vanillin ke dalam formulasi gel yang mengandungi minyak pati *P. aduncum* dapat meningkatkan keberkesanannya sebagai repelan terhadap *Ae. aegypti*.

Kata kunci: Repelan; *Piper aduncum*; *N,N*-dietil-3- metilbenzamida; gel; vanillin; minyak parafin

INTRODUCTION

Mosquitoes transmit diseases to more than 700 million individual each year and remain as a major source of illness and mortality worldwide (Govindarajan 2014). Dengue fever (DF) was one of the main life-threatening viral infections without vaccine or appropriate treatment (Patel et al. 2012). Therefore, personal protection method such as repellent has been used widely to prevent the transmission of mosquito borne diseases by minimizing the human-vector contact (Fradin & Day 2002). Personal protection methods remain as a crucial form of prevention for avoiding

mosquito-borne diseases due to absence of vaccine and specific treatment for most of the mosquito borne diseases (Bissinger & Roe 2010; Impoinvil et al. 2007).

Repellent is a chemical substances that act in vapor phase and prevent insects from biting or reaching the targets to which its attracted to (Browne 1977). Chou et al. (1997) referred repellent as a chemical substance that can inhibit mosquito host-seeking behavior in a vapor phase. Mosquitoes were attracted to the warmth, humid, carbon dioxide, odor and estrogen that were released by human skin (Brown 1966; Maibach et al. 1974). When repellent was applied on skin or cloth it will create a vapor

layer with an odor that was offensive to insects (Garson & Winnike 1968).

N,N-diethyl-3-methylbenzamide, DEET has been referred to as a gold standard repellent since its commercialization with protection time of 8 hours after application (McCabe et al. 1954). DEET based repellent can be found in various concentrations and can act as a strong molecular confusant by disturbing the activity of insects olfactory receptor (Bohbot & Dickens 2010; Pellegrino et al. 2011). However DEET has some adverse effects such as allergic reaction and other toxicity effects towards consumer (Qui et al. 1998). Toxicity due to DEET has been reported especially among childrens and elderly (Clem et al. 1993; Qiu et al. 1998; Sudakin & Trevathan 2003). It also cause contact urticaria, skin eruption or toxic encephalopathy in children (Thavara et al. 2002; Peterson & Coats 2001; Das et al. 2003). DEET was recommended for use with caution due to its ability to damage plastics and synthetic fabric and other toxic effect such as dermatitis, allergy and neurology and cardiovascular effect that was reported mainly due to misapplication of DEET (Katz et al. 2008; Peterson & Coats 2001; Osimitz et al. 2010).

Adverse effects due to synthetic repellent has lead to a study to find an effective, safer and inexpensive plant-based repellent as an alternative repellent (Abu-Qare & Abou-Donia 2001; Barnard & Xue 2004; Fradin 1998). Usage of plant essential oil (EO) as a potential mosquito controlling agents has been recognized and several studies conducted on their larvicidal and repellency potency have shown promising results (Sukumar et al. 1991; Shaalan et al 2005; Conti et al. 2010; Maia & Moore 2011; Zahran & Abdelgaleil 2011; Evergetis et al. 2012).

Piper aduncum Linnaeus, also known as *P. angustifolium*, *P. celtidifolium*, *P. elongatum*, *P. multinervium* and *P. stevensonii* (Burger 1971; Ciccio & Ballestro 1997; Olander et al. 1998), is a shrub with alternate leaves and spiky flowers. Its height can reach up to 7 to 8 meters. *P. aduncum* has traditionally been used in medicinal and culinary applications, and its EO is a well-known insecticide and molluscicide, in addition of having antibacterial activity (Pohlit et al. 2006). A previous study demonstrated that *P. aduncum* extract is effective against adult *Aedes aegypti* Linn. (Hidayatulfathi et al. 2004). According to Misni et al. (2008), *P. aduncum* EO has the ability to prevent mosquito bites thus can be develop as a mosquito repellent. However, most of the plant-based repellent provides short duration of protection against mosquito bites due to the volatility of its active ingredients. Several methods have been carried out to improve the short duration repellency of plant EO. One of them is through combination of several fixatives such as kerosene, vanillin, olive oil and tamanu oil (Traboulsi et al. 2005; Kamsuk et al. 2007; Moore et al. 2007; Hieu et al. 2010).

In this study, the *P. aduncum* EO was formulated into gel formulation containing vanillin and paraffin oil in various concentrations. The efficacy of the gel formulations were then tested against *Ae. aegypti* in the laboratory in

order to find the most suitable formulations that can prolong the efficacy of *P. aduncum* EO as repellent. Vanillin is a well-known fixative that able to improve the repellency activity of not only plant EO but also DEET (Tuetun et al. 2005; Tawatsin et al. 2001; Choochote et al. 2007). Other than vanillin, fixatives substances such as paraffin oil also may increase the repellent activity of plant EO (Oyedele et al. 2002).

MATERIALS & METHODS

CHEMICALS

Chemical used in this study was triethanolamine that was obtained from R&M Chemicals (UK). Paraffin oil and vanillin was obtained from Sigma-Aldrich (Germany) while tween 80 was from Sigma-Aldrich (USA). Carbopol 940 and DEET was supplied by Acros Organics (USA) and ethanol was from Merck (Germany). Anhydrous magnesium sulfate was obtained from Fisher Scientific (UK).

PIPER ADUNCUM ESSENTIAL OIL EXTRACTION

The leaves of *P. aduncum* were obtained from Batu 13 Gombak, Selangor, Malaysia, and the species was confirmed by the Malaysian Forest Research Institute (FRIM). The EO was extracted using a hydro-distillation method (FRIM 2000 unpublished data), and dried over anhydrous magnesium sulfate to obtain the EO used in the experiments. The voucher number for this plant is UKM b 29778 and was obtained from National University of Malaysia (UKM).

FORMULATIONS PREPARATION

Gel formulation base are consist of carbopol 940 (1.5% w/v), distilled water, tween 80 and triethanolamine. Five gel formulations containing 10% *P. aduncum* with different fixatives and other added excipients was prepared and designated as A, B, C, D and E (Table 1). The gel formulations was prepared by dispersing 1.5 g of carbopol 940 in 100 ml distilled water under continuous stirring until homogenous dispersion was formed. After that, other ingredients were added into the gel formulations as in Table 1. These formulations were then compared with DEET (10% w/w) only in gel formulations as a positive control.

EXPERIMENTAL MOSQUITOES

Ae. aegypti mosquitoes (WHO susceptible strain), provided by the Institute for Medical Research (IMR, Jalan Pahang 50588, Kuala Lumpur, Malaysia), were reared in an insectarium of the Department of Biomedical Science, UKM, under laboratory conditions (25-30°C, R.H. 60-70%). Adult mosquitoes were maintained in screened cages and fed on 10% sucrose solution (in water).

REPELLENT TESTING

Testing was conducted based on the protocol *Household insecticide products-personal mosquito repellent – evaluation method for biological efficacy* (MS1497:2007) provided by the Standard and Industrial Research Institute of Malaysia (SIRIM). Four human subjects were used for each *P. aduncum* gel formulations and positive control, DEET (10% w/w). The test was carried out in triplicate for each subject for all formulations tested. Each subject served as both test and negative control, with the EO gel formulation or DEET (positive control) tested on one hand and the negative control tested on the other hand. For example, for a trial of formulation A, the subject had formulation A applied to one hand and negative control for formulation A (gel only) to the other. Each gel formulations (A, B, C, D and E) has their own negative control formulations (gel formulations without *P. aduncum* EO). The subjects were UKM students, females age 20-30 years old, who agreed to take part in the study. This study was approved by the UKM Human Ethics Committee (UKM 1.5.3.5/244/NN-143-2011). Testing was carried out during *Ae. aegypti* biting time (0800-1300). An area of 3 cm x 8 cm was drawn on each of the subject's hands, and 0.5 g repellent formulation (either *P. aduncum* EO or DEET) was applied within this area on one hand while the negative control formulation was applied to the subject's other hand in the delineated area. The subject's hands were then inserted into a 60 cm x 60 cm x 60 cm test cage with a circular opening containing 25 nulliparous female *Ae. aegypti* (5 to 7 days old) that had fasted overnight. The subjects' hands were covered with rubber gloves and only the treated area was exposed for mosquito biting. The number of mosquito landing/or biting within 5 minutes was calculated and recorded. This procedure was repeated every 30 minutes until 4 hours post-application. Repellency percentage was calculated as follows:

$$\frac{C-T}{C} \times 100, \quad (1)$$

from which C = Number of mosquitoes on negative control arm and T = Number of mosquitoes on repellent treated arm.

STATISTICAL ANALYSIS

Statistical analysis was conducted using IBM SPSS Statistics software. The data was normally distributed thus proceed with a split-plot ANOVA (SPANOVA) test to analyze a statistical significance for repellency percentage of all gel formulations and DEET. Level for significance was set at $p < 0.05$.

RESULTS

Results of repellency percentage of gel formulations containing *P. aduncum* EO and DEET was shown in Table 1. Immediately after application, all gel formulations were able to provide adequate protection against mosquito bites with repellency percentage ranging from 99% to 100%. After 60 min post-application, repellency percentage of formulation C start to decrease while other formulations were still maintaining their repellency effect > 90%. At 180 min post-application, formulation D and E was able to maintain their repellency effect > 85% while formulation A, B and C showed < 60% repellency. After 240 min post-application, formulation D and E was able to provide >70% repellency percentage against mosquito while formulation A, B and C gives < 50% repellency which are 43.5%, 30.8% and 29.6% respectively. The order of efficacy for the gel formulations was Formulation E > Formulation D > Formulation A > Formulation B > Formulation C. Positive control, DEET in the same concentrations gave higher repellency effect within 4 h application compared to gel formulations containing *P. aduncum* EO. DEET at 240 min post-application was able to provide 92.3% repellency percentage. There were significant difference in repellency percentage between gel formulations and DEET ($F_{4,21} = 20.696, p = 0.001$).

TABLE 1. Gel formulations containing 10% *P. aduncum* essential oil

Formulations	Content				
	<i>Piper aduncum</i> (w/w)	Vanillin (w/w)	Paraffin oil (w/w)	Aloe vera (w/w)	Vitamin E (w/w)
A	10%	-	-	-	-
B	10%	-	-	1%	1%
C	10%	-	6%	1%	1%
D	10%	5%	-	1%	1%
E	10%	10%	-	1%	1%

DISCUSSION

Adverse effect of synthetic repellent especially DEET has led to numerous studies regarding plant as a potential alternative of synthetic repellent. It was believed that plant-

based products do not pose any toxicity effect to human or animals and it is biodegradable (Das et al. 2003; Chogo & Crank 1981). However, plant-based repellent provided a short-lived efficacy due to high volatility properties of the active ingredients. One of the method that can be use to

TABLE 2. Repellency percentage of *P. aduncum* essential oil in different gel formulations and DEET

Time/min		Formulations					
		A	B	C	D	E	10% DEET
0	Control	40.5±12.7	23.0±3.6	4.3±3.0	11.0±7.0	12.8±7.4	52.0±1.8
	Test	0.3±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Repellency percentage (%)	99.5	100	100	100	100	100
30	Control	55.0±5.8	65.0±16.8	22.8±3.5	22.0±15.6	15.3±6.2	57.5±2.9
	Test	0.8±1.5	1.0±0.8	0.3±0.5	0.0±0.0	0.0±0.0	0.0±0.0
	Repellency percentage (%)	98.8	98.6	99	100	100	100
60	Control	59.3±12.9	65.8±17.2	32.3±9.7	34.5±25.1	25.5±8.8	62.3±3.6
	Test	4.0±2.5	4.5±2.6	12.0±6.1	1.0±1.2	0.0±0.0	0.0±0.0
	Repellency percentage (%)	93.6	93.4	64.2	97.1	100	100
90	Control	64.3±7.6	68.3±12.4	41.8±18.1	37.8±22.3	23.3±7.5	67.3±1.9
	Test	6.5±1.3	17.0±8.4	20.3±14.5	1.5±1.7	0.3±0.5	0.0±0.0
	Repellency percentage (%)	89.7	76.2	54.6	96.4	99.2	100
120	Control	62.3±6.6	69.3±11.5	49.3±19.8	40.3±25.1	23.5±11.7	72.5±2.5
	Test	16.5±3.5	30.3±12.4	27.3±21.4	3.5±2.5	0.5±1.0	0.0±0.0
	Repellency percentage (%)	73.7	57.8	50.4	91.7	93.8	100
150	Control	61.5±5.2	72.8±4.5	52.8±19.7	36.5±28.8	31.3±15.5	73.8±2.5
	Test	18.3±3.9	35.5±15.1	31.0±22.6	6.5±5.5	1.3±1.5	2.0±2.5
	Repellency percentage (%)	70.2	52	45.3	81.9	96.8	97.3
180	Control	56.5±12.2	73.3±3.5	58.8±16.5	42.8±30.3	28.5±12.6	75.0±0.0
	Test	21.5±3.1	39.3±14.4	34.8±23.5	6.8±5.3	3.0±1.4	3.3±3.9
	Repellency percentage (%)	59.5	47	44	87.3	88.1	95.7
210	Control	54.0±11.3	73.8±2.5	60.3±11.1	43.5±26.6	29.3±18.3	75.0±0.0
	Test	19.5±0.6	43.3±14.5	40.8±23.6	7.3±5.2	5.0±3.4	4.8±5.6
	Repellency percentage (%)	62.3	41.8	35.5	86.2	77.6	93.7
240	Control	51.8±15.7	74.8±0.5	61.8±8.9	42.5±28.3	30.0±10.6	75.0±0.00
	Test	27.0±2.9	51.8±6.1	44.8±24.4	10.8±6.7	7.3±7.9	5.8±6.9
	Repellency percentage (%)	43.5	30.8	29.6	71.8	77.9	92.3

enhance the protective effects of plant-based repellent is by developing a formulation that would fix the aromatic constituents of the EO on skin for an appropriate time, thus increase the efficacy of the EO close to or exceeds the efficacy of DEET (Rozendaal 1997).

Fixatives refer to a substance that has the ability to increase the intensity and lasting qualities of aromatic components of fragrances materials (Seldner 1982). They have lower volatility properties compare to fragrances and have the ability to slow the evaporation of volatile oils (Dobbs et al. 2009; Songkro et al. 2012). Addition of fixatives into formulation was considered as the simplest method to enhance the repellency activity of certain active ingredients compared to other formulation techniques such as microcapsule and nanoemulsion methods (Champakaew et al. 2016).

In this study, the efficacy of five gel formulations containing *P. aduncum* EO with different fixative was investigated in order to determine the most appropriate fixative that can increase the efficacy of the EO as repellent. In this study it was proven that vanillin can prolong the efficacy of *P. aduncum* EO against *Ae. aegypti*. The addition of vanillin into gel formulations containing

P. aduncum EO (formulation D and E) were able to prolong the effect of the EO as repellent compared to other gel formulations (formulation A, B and C). Formulation D and E provided >70% repellency percentage for 4 h. Study done by Tawatsin et al. (2001) also found that the addition of 5% vanillin to turmeric and hairy basil EO was able to prolong the repellency effect of the EO (Tawatsin et al. 2001). Yang and Ma (2005) also stated that the protection time of *Eucalyptus globules* alcoholic solution against *Ae. albopictus* can be improved by adding a 5% of vanillin. Study by Thomas et al. (2009) found that the addition of 5% vanillin did not promote the repellent activity of *Kunzea ambigua* EO, an Australian Native plant. However, in this study, the addition of 5% vanillin was able to prolong the effect of *P. aduncum* EO gel formulations. Songkro et al. (2012) reported that addition of 5% vanillin to citronella oil lotions increase the protection time against *Ae. aegypti* for 1.3 hour to 4.8 h. The addition of 5% vanillin was able to prolong the protection time of Angelica sinensis (Oliv.) hexane extract (AHE) against *Ae. aegypti* (Champakaew et al. 2016). Choochote et al. (2007) has tested a volatile oils mixed with 10% vanillin against *Ae. aegypti* in laboratory and found that vanillin was able to increase the efficacy

of the EO as repellent. Addition of vanillin to *Zanthoxylum piperitum* EO induces repellency effect on *Armigeres subalbatus*, a species that is tolerant to DEET (Kamsuk et al. 2007).

However, unlike vanillin, the addition of 6% paraffin oil (formulations C) into the gel bases didn't increase the efficacy of the *P. aduncum* EO as the repellency percentage was similar to non-fixative formulations (formulation A and B). The repellency percentage of all gel formulations was significantly lower than DEET. At the same concentration (10%), DEET provided 100% repellency > 2 h post-application. DEET based-repellent was able to remain effective up to 8 to 12 h from the time of application even under harsh climatic conditions (Logan et al. 2010). Study by Masetti & Maini (2006) found that DEET-based repellent (Off Spray) containing 15% DEET gave 4 h protection time whereas Frances et al. (2005) in their study found that OFF Skintastic product containing 7% DEET and Aerogard containing 12% DEET provided 2 to 5 h protection against all mosquitoes including *Ae. aegypti*.

CONCLUSION

From the present study, we found that the repellency percentages of the *P. aduncum* EO gel formulations containing fixatives were generally longer than that of the formulation that did not contain any fixatives. However, DEET still provides excellence repellency activity against *Ae. aegypti* compared to *P. aduncum* EO gel formulations. Nevertheless, *P. aduncum* gel formulations with vanillin were able to provide adequate protection against mosquito bites. Gel formulations containing vanillin displayed better repellent properties (repellency percentage > 85% for 180 min post-application) than gel formulations containing paraffin oil. Therefore, they are the most promising vehicle for *P. aduncum* EO and can be used for further development and commercialization as an alternative to synthetic repellents.

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REFERENCES

- Abu-Qare, A.W. & Abou-Donia, M.B. 2001. Development of a high-performance liquid chromatographic method for the quantification of chlorpyrifos, pyridostigmine bromide, N,N-diethyl-m-toluamide and their metabolites in rat plasma and urine. *Journal of Chromatography. Biomedical Sciences and Applications* 754(2): 533-538.
- Barnard, D.R. & Xue, R.D. 2004. Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripalpus* and *Ochierotatus triseriatus* (Diptera: Culicidae). *Journal of medical entomology* 41(4): 726-730.
- Bissinger, B.W. & Roe, R.M. 2010. Tick repellents: Past, present, and future. *Pesticide Biochemistry and Physiology* 96(2): 63-79.
- Brown, A.W.A. 1966. The attraction of mosquitoes to hosts. *The Journal of the American Medical Association* 196(3): 249-252.
- Browne, L.B. 1997. Host-related responses and their suppression: some behavioral considerations. In: Shorey, H.H. & McKelvey, J.J. Jr (Eds.) *Chemical Control of Insect Behavior*, pp. 117-127. New York: Wiley.
- Bohbot, J.D. & Dickens, J.C. 2010. Insect repellents: Modulators of mosquito odorant receptor activity. *PLoS One* 5(8): 121-138.
- Burger, W.C. 1971. Piperaceae. *Fieldiana (Botany)* 35: 79-96.
- Champakaew, D., Junkum, A., Chaithong, U., Jitpakdi, A., Riyong, D., Wannasan, A., Intirach, J., Muangmoon, R., Chansang, A., Tuetun, B. & Pitasawat, B. 2016. Assessment of *Angelica sinensis* (Oliv.) Diels as a repellent for personal protection against mosquitoes under laboratory and field conditions in northern Thailand. *Parasites & Vectors* 9(1): 373.
- Chogo, J.B. & Crank, G. 1981. Chemical composition and biological activity of the Tanzanian plant *Ocimum Suave*. *Journal of Natural Products* 44(3): 308-311.
- Choochote, W., Chaithong, U., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Tuetun, B., Champakaew, D. & Pitasawat, B. 2007. Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia* 78(5): 359-364.
- Chou, J.T., Rossignol, P.A. & Ayres, J.W. 1997. Evaluation of commercial insect repellents on human skin against *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* 34(6): 624-630.
- Ciccio, J.F. & Ballester, C.M. 1997. Constituyentes volátiles de las hojas y espigas de *Piper aduncum* (Piperaceae) de Costa Rica. *Revista de Biología Tropical* 45: 783-790.
- Clem, J.R., Havemann, D.F. & Raebel, M.A. 1993. Insect repellent (N,N-diethyl-m-toluamide) cardiovascular toxicity in an adult. *The Annals of pharmacotherapy* 27(3): 289-293.
- Coleman, R.E., Robert, L.L., Roberts, L.W., Glass, J.A., Seeley, D.C., Laughinghouse, A., Perkins, P.V. & Wirtz, R.A. 1993. Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: Culicidae) and two phlebotomine sand flies (Diptera: Psychodidae). *Journal of Medical Entomology* 30: 499-502.
- Conti, B., Canale, A., Bertoli, A., Gozzini, F. & Pistelli, L. 2010. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitology Research* 107(6): 1455-1461.
- Das, M.K. & Ansari, M.A. 2003. Evaluation of repellent action of *Cymbopogon martinii* Stapf var *sofia* oil against *Anopheles sundaicus* in tribal villages of Car Nicobar Island, Andaman & Nicobar Islands, India. *Journal of Vector Borne Diseases* 40(3-4): 101-104.
- Dobbs, S.W., Oldfield, T.A. & Olsen, D.J. 2009. Fragrance fixatives. US Application Publication no. US 2009/0165360 A1

- Evergetis, E., Michaelakis, A., Haroutounian, S.A. 2012. Essential oils of Umbelliferae (Apiaceae) family taxa as emerging potent agents for mosquito control. In: Larramendy, M.L. & Soloneski, S. (Ed). *Integrated Pest Management and Pest Control- Current and Future Tactics*, pp 613-638. InTech.
- Fradin, M.S. 1998. Mosquitoes and Mosquito Repellents A Clinician's Guide. *Annals of Internal Medicine* 128: 931-940.
- Fradin, M. S. & Day, J. F. 2002. Comparative efficacy of insect repellents against mosquito bites. *New England Journal of Medicine* 347(1): 13-18.
- Frances, S.P. & Wirtz, R.A. 2005. Repellents: past, present, and future. *Journal of the American Mosquito Control Association* 21(4): 1-3.
- Garson, L.R. & Winnike, M.E. 1968. Relationship between insect repellency and chemical and physical parameters: A review. *Journal of Medical Entomology* 5: 339-352.
- Hieu, T.T., Kim, S.I., Kwon, H.W. & Ahn, Y.J. 2010. Enhanced repellency of binary mixtures of *Zanthoxylum piperitum* pericarp steam distillate or *Zanthoxylum armatum* seed oil constituents and *Calophyllum inophyllum* nut oil and their aerosols to *Stomoxys calcitrans*. *Pest Management Science* 66(11): 1191-1198.
- Hidayatulfathi, O., Sallehuddin, S. & Ibrahim, J. 2004. Adulticidal activity of some Malaysian plant extracts against *Aedes aegypti* Linnaeus. *Tropical Biomedicine* 21(2): 61-67.
- Impoinvil, D.E., Ahmad, S., Troyo, A., Keating, J., Githeko, A.K., Mbogo, C.M., Kibe, L., Githure, J.I., Gad, A.M., Hassan, A.N., Orshan, L., Warburg, A., Calderon-Arguedas, O., Sanchez-Loria, V.M., Velit-Suarez, R., Chadee, D.D., Novak, R.J., Beier, J.C., 2007. Comparison of mosquito control programs in seven urban sites in Africa, the Middle East and the Americas. *Health Policy* 83(2-3): 196-212.
- Kamsuk, K., Choochote, W., Chaithong, U., Jitpakdi, A., Tippawangkosol, P., Riyong, D. & Pitasawat, B. 2007. Effectiveness of *Zanthoxylum piperitum*-derived essential oil as an alternative repellent under laboratory and field applications. *Parasitology Research* 100(2): 339-345.
- Katz, T.M., Miller, J.H. & Hebert, A.A. 2008. Insect repellents: historical perspectives and new developments. *Journal of the American Academy of Dermatology* 58(5): 865-871.
- Korthou, H. & Verpoorte, R. 2007. Vanilla. In Berger, R.G. (ed.). *Flavours and fragrances: chemistry, bioprocessing and sustainability*, pp. 203-217. Heidelberg, Germany: Springer.
- Logan, J.G., Stanczyk, N.M., Hassanali, A., Kemei, J., Santana, A.E.G., Ribeiro, K.A.L., Pickett, J.A. & Mordue Luntz, A.J. 2010. Arm-in-cage testing of natural human-derived mosquito repellents. *Malaria Journal* 9(1): 239.
- Govindarajan, M. 2014. Mosquito repellent properties of *Delonix elata* (L.) gamble (Family: Fabaceae) against filariasis vector, *Culex quinquefasciatus* Say. (Diptera: Culicidae). *Asian Pacific Journal of Tropical Disease* 4(1): 194-198.
- Maia, M.F. & Moore, S.J. 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal* 10(1): S11.
- Maibach, H.I., Khan, A.A. & Akers, W.A. 1974. Use of insect repellents for maximum efficacy. *Archives of Dermatology* 109(1): 32-35.
- Masetti, A. & Maini, S. 2006. Arm in cage tests to compare skin repellents against bites of *Aedes albopictus*. *Bulletin of Insectology* 59: 157-160.
- McCabe, E.T., Barthel, W.F., Gertler, S.I. & Hall, S.A. 1954. Insect repellents. III. N,N-diethylamides. *The Journal of Organic Chemistry* 19: 493-498.
- Misni, N., Sulaiman, S. & Othman, H. 2008. The repellent activity of *Piper aduncum* Linn (Family: Piperaceae) essential oil against *Aedes aegypti* using human volunteers. *Journal of Tropical Medicine & Parasitology* 31: 63-69.
- Moore, S.J., Darling, S.T., Sihuincha, M., Padilla, N. & Devine, G.J. 2007. A low-cost repellent for malaria vectors in the Americas: results of two field trials in Guatemala and Peru. *Malaria Journal* 6: 101.
- Olander, L.P., Scatena, F.N. & Silver, W.L. 1998. Impacts of disturbance initiated by road construction in a subtropical cloud forest in the Luquillo Experimental Forest, Puerto Rico. *Forest Ecology and Management* 109: 33-49.
- Osimitz, T.G., Murphy, J.V., Fell, L.A. & Page, B. 2010. Adverse events associated with the use of insect repellents containing N,N-diethyl-m-toluamide (DEET). *Regulatory Toxicology and Pharmacology* 56(1): 93-99.
- Oyedele, A.O., Gbolade, A.A., Sosan, M.B., Adewoyin, F.B., Soyelu, O.L. & Orafidiya, O.O. 2002. Formulation of an effective mosquito-repellent topical product from lemongrass oil. *Phytomedicine* 9(3): 259-262.
- Patel, E.K., Gupta, A. & Oswal, R.J. 2012. A review on: mosquito repellent methods. *International Journal of Pharmaceutical, Chemical and Biological Sciences* 2(3): 310-317.
- Pellegrino, M., Steinbach, N., Stensmyr, M.C., Hansson, B.S. & Vossball, L.B. 2011. A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor. *Nature* 478: 511-514.
- Peterson, C. & Coat, J. 2001. Insect repellents - past, present and future. *Pesticide Outlook* 12: 154-158.
- Pohlit, A.M., Pinto, A.C.S. & Mause, R. 2006. *Piper aduncum* L.: pluripotent plant and important phytochemical substance source. *Revista Fitos* 2: 7.
- Qiu, H., Jun, H.W. & McCall, J.W. 1998. Pharmacokinetics, formulation, and safety of insect repellent N,N-diethyl-3-methylbenzamide (DEET): A review. *Journal of the American Mosquito Control Association* 14(1): 12-27.
- Rozendaal, J.A. 1997. *Vector control: methods for use by individuals and communities*. Geneva: World Health Organization.
- Seldner, A. 1982. *Polyol fragrance fixatives*. U.S. patent. Standard and Industrial Research Institute of Malaysia. 2007. Household insecticide products-personal mosquito repellent – evaluation method for biological efficacy (MS1497:2007).
- Sudakin, D.L. & Trevathan, W.R. 2003. DEET: A review and update of safety and risk in the general population. *Journal of Toxicology: Clinical Toxicology* 41(6): 831-839.
- Songkro, S., Jenboonlap, M., Boonprasertpon, M., Maneenuan, D., Bouking, K. & Kaewnopparat, N. 2012. Effects of glucam P-20, vanillin, and fixolide on mosquito repellency of citronella oil lotions. *Journal of Medical Entomology* 49(3): 672-677.
- Sukumar, K., Perich, M.J. & Boobar, L.R. 1991. Botanical derivatives in mosquito control: A review. *Journal of the American Mosquito Control Association* 7(2): 210-237.
- Shalan, E.A.S., Canyon, D., Younes, M.W.F., Abdel-Wahab, H. & Mansour, A.H. 2005. A review of botanical phytochemicals with mosquitocidal potential. *Environment International* 31: 1149-1166.

- Sweetman, S. 2005. *Martindale: the Complete Drug Reference*. London, United Kingdom: Pharmaceutical Press.
- Traboulsi, A.F., El-Haj, S., Tueni, M., Taoubi, K., Nader, N.A. & Mrad, A. 2005. Repellency and toxicity of aromatic plant extracts against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). *Pest Management Science* 61(6): 597-604.
- Thavara, U., Tawatsin, A. & Chompoosri, J. 2002. Phytochemicals as repellents against mosquitoes in Thailand. *Proceedings International Conference on Biopesticide* 3, pp. 244-250.
- Tawatsin, A., Wratten, S.D., Scott, R.R., Thavara, U. & Techadamrongsin, Y. 2001. Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology* 26(1): 76-82.
- Thomas, J., Webb, C.E., Narkowicz, C., Jacobson, G.A., Peterson, G.M., Davies, N.W. & Russell, R.C. 2009. Evaluation of repellent properties of volatile extracts from the Australian native plant *Kunzea ambigua* against *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* 46(6): 1387-1391.
- Walker, T.W., Robert, L.L., Copeland, A., Githeko, A.K., Wirtz, R.A., Githure, J.I. & Klein, T.A. 1996. Field evaluation of arthropod repellents, deet and a piperidine compound, AI3-37220, against *Anopheles funestus* and *Anopheles arabiensis* in West Kenya. *Journal of the American Mosquito Control Association* 12: 172-176.
- Walton, N.J., Mayer, M.J. & Narbad, A. 2003. Vanillin. *Phytochemistry* 63(5): 505-515.
- Yang, P. & Ma, Y. 2005. Repellent effect of plant essential oils against *Aedes albopictus*. *Journal of Vector Ecology* 30: 231-234.
- Zahran, H.E.D.M. & Abdelgaleil, S.A.M. 2011. Insecticidal and developmental inhibitory properties of monoterpenes on *Culex pipiens* L. (Diptera: Culicidae). *Journal of Asia-Pacific Entomology* 14: 46-51.
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